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Detection of respiratory viral antigens in cattle lung tissues by direct ELISA

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Abstract: Bovine Respiratory Disease is one of the most important diseases with serious financial losses for the cattle industry in worldwide. The aim of this study was to detect the associations between respiratory viruses; bovine herpes virus 1, bovine viral diarrhea virus, bovine respiratory syncytial virus, and Para influenza virus 3 statuses of a herd and bovine respiratory disease occurrence. Present study describes virological distribution of bovine respiratory viruses in non-vaccinated cattle (for mentioned infections) of Central Anatolia, Turkey. A total of 24 lung tissue samples were collected during the December 2012 to January 2013 from cattle that died after manifesting clinical signs of respiratory system. Samples were successfully homogenized. Tissue samples were analyzed for detecting antigens by commercially available direct ELISA kit. BRSV antigens were detected in lung tissues 4 out of 24 tested cattle with a percentage of 16.6%, whereas BHV-1, BVDV and PI-3 were not found. BRSV may be common reason of respiratory diseases in herds. It has been also offered advice about prevention of respiratory viral infection for health planning. In conclusion, existence of BRSV infection is still defined and may play an important role in the respiratoric viral infection of cattle.

Keywords: BHV-1, BRSV, BVDV, PI-3, ELISA

1. Introduction

Bovine Respiratory Disease (BRD) infection occurs in cattle in different regions of the world [1,2]. Respiratory viruses such as *bovine herpes virus 1* (BHV-1), *bovine viral diarrhea virus* (BVDV), *bovine respiratory syncytial virus* (BRSV), and *Para influenza virus 3* (PI-3) are the most important disease for cattle industry in worldwide [3].

BHV-1 is a pathogen of cattle associated with two major syndromes, called infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV), it is characterized by abortion, encephalitis fatal disease in newborn calves, mastitis, tracheitis, and thus causes great economic losses to the young and adult cattle industry [4,5]. It is a member of the family *Herpesviridae*, subfamily *Alphaherpesvirinae* [6].

BVDV causes a disease in cattle, and characterized with respiratory and reproductive symptoms, abortions, mummification, congenital anomalies, still-births, and birth of persistently infected (PI) carrier animals, and can lead to fatal mucosal disease [7,8]. BVDV is a *Pestivirus* in the *Flaviviridae* family and is closely related to Classical Swine Fever and Border Disease Virus [9]. BVDV strains are divided into two biotypes such as noncytopathic (ncp) and cytopathic (cp) according to proliferation in cell culture [10].

BRSV infection is characterized with rhinitis, coughing, abdominal breathing, bronchiolitis, reduced appetite, interstitial edema, emphysema and some cases progressing to sever bronchopneumonia may end with death [11]. It is a member of the family *Paramyxoviridae*, genus *Pneumovirus*. BRSV genome is negative sense, single stranded, non-segmented RNA, and 15.2 kb long [12]. Serological tests such as Enzyme Linked Immunosorbent Assay (ELISA), Complement Fixation Test (CFT), and Serum Neutralization Test (SNT) are useful for BRSV diagnosis [13].

PI-3 is a member of the family *Paramyxoviridae*, subfamily *Paramyxovirus*. PI-3 is an enveloped, non-segmented, negative-sense RNA virus. It is characterized with acute and silently fever, rhinitis, nasal secretions [14]. PIV-3 causes generally subclinical infections and clinical symptoms but with secondary bacterial and other viral infections may lead death [15].

Diagnosis of major viral causes of bovine respiratory infections has been based largely on the serological testing by Virus Neutralization Test (VNT), ELISA, Immunofluorescence Antibody (IFA), Hem-agglutination Inhibition (HI), and nested Polymerase chain reaction [16]. Detecting and controlling bovine viral respiratory diseases can decrease economic losses [17].

The present study was planned to detect the associations between respiratory viruses; BHV-1, BVDV, BRSV, and PI-3 status of a herd and BRD occurrence using ELISA.

2. Materials and Methods

A total of 24 lung tissue samples were collected during the December 2012 to January 2013 from dead cattle (2-3 years) for the presence of respiratory viral antigens in a dairy cattle herd (250 capacity) in Konya, Central Anatolia region of Turkey. Individual samples of lung tissues (approximately 1 gram) were collected max 6 hours after dead during necropsy by Veterinary and successfully homogenized with 2 ml of lysis solution and centrifuged at 500 rpm for 10 min to obtain the supernatant. All applications were performed under sterile conditions. Tissue samples were analyzed for detecting mentioned viral antigens by a commercially available direct ELISA kit (Biox Diagnostics, BIO K 340/2, Belgium) in Virology laboratories of Veterinary Medicine Faculty of Selcuk University. The test was performed as per the manufacturer's instructions. The plates were then read on an automatic micro plate reader (Rayto RT 2100C, China) at 450 nm. ELISA results were calculated for each sample the S/P ratio (in %):

$$\frac{S}{P(\%)} = \frac{OD_{sample} * 100}{OD_{Positive\ Control}}$$

Samples were considered positive for an S/P (%) > 6.94 for BHV-1, > 7.50 for BVDV, > 6.99 for BRSV, and > 6.93 for PI-3. Statistical significance of differences between viral infections was analyzed using Chi-square analysis (Minitab 14.0 Inc., State College, PA, USA). $P < 0.05$ level was accepted statistically significant.

3. Results

Table 1. *Direct ELISA results of lung tissue*

Infection	ELISA-Ag
BHV-1	$0/24^{b}$ (Not detected)
BVDV	$0/24^{b}$ (Not detected)
PI-3	$0/24^{b}$ (Not detected)
BRSV	$4/24^{a}$ (16.6%)

[a, b,]: Values marked with different letters in the same column are statistically significant ($P<0.05$).

Specific BRSV antigens were detected in 4 out of the 24 (16.6%) tested cattle lung samples (Table 1). No positive sample was obtained for other viruses (BHV-1, BVDV and PI-3).

4. Discussion

Bovine respiratory diseases are the most important diseases affecting respiratory tract both young and adult cattle in worldwide [18]. The primary viral respiratory pathogens are BHV-1, BRSV, and PI-3 in cattle [19]. In this study, a total of 24 lung tissue samples were collected from died cattle after manifesting clinical signs of respiratory system in Central Anatolia. Specific BRSV antigen was detected in 4 lung tissue sample out of 24 tested samples with a percentage of 16.6% and there were no positive samples for other infections.

The serologic and virologic evidence of infections caused by respiratory viruses in Turkey has been demonstrated different studies previously [20,21]. The serologic evidence of BRSV infections in Turkey [20,21] and other countries [22,23] has been demonstrated different studies previously. It is known that lung tissue samples containing high amount of BRSV antigens [24]. Also, most cases of BRSV infections occurring in late autumn and winter [25]. The reason of only BRSV antigens detected in this study can be explained by sampled season and tissues.

It is reported that virus isolation and fluorescent antibody test (FAT) are more sensitive and simple for antigen detection than ELISA in the diagnosis of BRSV infection [26]. However, the ability to perform ELISA within a short time frame to detect different viral agents reduces hands-on time in the laboratory, is more efficient in differential diagnosis [27]. In this study, the samples have no examined by FAT or isolation so there is not evaluated the specificity of other tests. But detection of BRSV antigen by direct ELISA, it can be played an important role of cattle dead.

In consideration of the characteristic of *herpesvirus* infections to establish latency, it couldn't detect in lung samples [28]. In the current study, all lung samples were detected as negative for PI-3. So it has not been evaluated the reason of death. It has been reported that causes generally subclinical infections and clinical symptoms but with secondary bacterial and other viral infections may lead death [15].

BHV-1, BVDV, BRSV and PI-3 infections in cattle have been reported by different researchers using serologically methods [20,29,30]. The current study would suggest that cattle may dead when exposed to BRSV. Infection with BHV-1, BRSV and PI-3 can also facilitate invasion of secondary pathogens such as *Pasteurella multocida*, *Haemophilus somni*, *Mycoplasma bovis* and *Mycoplasma dispar* [31]. Herd capacity [21], herd management strategies and environmental conditions are highly related to BRDV infections [32]. In this study, samples were not investigated for other potential bacterial agents so it has not been considered for mix infections.

5. Conclusion

In conclusion, the results showed the presence of BRSV infection among cattle in Central Anatolia of Turkey. For

control of the BRSV and other respiratory diseases, serological and virological examination should be done and prevention measures must be taken.

Competing Interests

The authors declare that they have no competing interests.

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